UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

January 31, 2018

MEMORANDUM

SUBJECT: Response to Bee Vectoring Technology's Response to EPA's Deficiencies Outlined in the Ecological Review and Environmental Risk Assessment of Section 3 Registration of the New End Use Product Vectorite containing *Clonostachys rosea* CR-7 vectored by honey and bumble bees. EPA File Symbol 90641-E. Decision # 520718.

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THRU: Shannon Borges, Senior Scientist
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TO: Nicola Steinmetz, Regulatory Action Leader
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ACTION REQUESTED: Review the response to EPA's deficiency letter from Bee Vectoring Technology regarding *C. rosea* CR-7 Vectorite.

CONCLUSION: The rationale response from the applicant is not adequate, because it did not address EPA's concerns and a larval bee study protocol and data from a larval study were not submitted. The rationale challenged EPA's position that *C. rosea* is an entomopathogen and the rationale provided arguments that bee hive temperature is too high for *C. rosea* growth, and the limited carrying capacity for bees of *C. rosea* limits the amount carried to flowers and bee hives. Published scientific research has shown that *C. rosea* is an entomopathogen for soft bodied insects and there is concern that due to the unique use pattern of Vectorite, *C. rosea* may be toxic/pathogenic to honey bee brood given the use pattern and increased delivery of spores to flowers which has been shown through increased efficacy relative to spray uses (BVT, 2018). Furthermore, C. *rosea* causes bee brood mortality at normal hive temperatures. Therefore, the rationale submitted in response to the deficiency letter is insufficient to address EPA's concerns, and a 21-day larval study as outlined by OECD (OECD, 2016) is required. Prior to conducting the study, Bee Vectoring Technology must submit a study protocol for formal review.

DATA REVIEW RECORD

Active Ingredient: *Clonostachys rosea* CR-7
Product Name: Vectorite
Company Name: Bee Vectoring Technology
EPA File Symbol: 90641-E
Submission Number: 990850
DP Barcode: 436548
MRID No: 50477501

The conclusion of EPA's deficiency letter sent to the applicant (U.S. EPA 2017a) stated that *C. rosea* is considered an entomopathogen and may have toxic/pathogenic effects on honey bee brood via increased exposure through the bee vectoring use pattern. Thus, EPA required a larval honey bee study as outlined by OECD (OECD, 2016). Prior to conducting the 21-day larval bee study, EPA recommended submission of a study protocol for review.

In response to the letter and to address concerns over potential hazard to bee larvae, the applicant provided a rationale including several literature citations and new data on bee carrying capacity of spores. In the ecological risk assessment (U.S. EPAb), the EPA cited several scientific publications that described *C. rosea* as a pathogen of arthropods and nematodes. These studies included Vega et al. (2008), which demonstrated pathogenicity of *C. rosea* in coffee berry borer; Zhang et al. (2008), which examined the nematicidal mode of action of *C. rosea*; and Ahmed et al. (2010), which examined the nematicidal efficacy of *C. rosea* in fecal pats of sheep pastures; Hamiduzzaman et al. (2012), which showed that *C. rosea* causes mortality to Varroa mites. According to personal communication with the first author of the last publication listed, *C. rosea* also causes mortality in bee brood (M. Hamiduzzaman, personal communication, January 11, 2018). Based on evidence in the literature, and because of the potential for increased exposure specifically to bee larvae, thus EPA has concerns that it will cause mortality in bee brood under the bee vectoring use pattern.

The applicant responded in MRID 50477501 (Tedford, 2017) that the Vega et al. 2008 publication on coffee berry borers had very small differences between the control and treatment groups and that the 0.5% sodium hypochlorite treatment of all insects prior to *C. rosea* inoculation may have predisposed the borers to colonization by *C. rosea* at 10^7 spores/ml. The response did not address the other studies mentioned in EPA's review and risk assessment with the exception of Hammiduzzaman et al. 2012. The applicant's response simply stated that the data showed it is a treatment for Varroa mites, which is accurate but did not provide any rebuttal. EPA agrees that the differences are small in the Vega et al. 2008 study but each of the borers tested was exposed to sodium hypochlorite and differences were observed between the control and the C. rosea test group, therefore the sodium hypochlorite did not have as great an effect as the treatments. Furthermore, since other studies indicate the potential for this fungus to penetrate invertebrate cuticle consisting of chitin, and based on additional information provided in follow-up communications, EPA still has concerns that *C. rosea* may cause adverse effects in honey bee larvae.

Bee Vectoring Technology also cited literature indicating that the optimal temperature for a honey bee hive is 35 °C with a range of 32 to 36 °C. The maximum growth for *C. rosea* is 33-36 °C. A growth curve was provided in the response for *C. rosea* CR-7 showing that vegetative growth was relatively low at 35 °C, while peak growth was at 30 °C. No information was provided regarding the temperature at which *C. rosea* CR-7 sporulates, and no information was provided on the vegetative growth between 30

°C and 35 °C. Therefore, it is not clear at what temperatures *C. rosea* CR-7 may sporulate and continue to grow in a bee hive, so the growth temperature is of limited application in making a risk determination for bee larvae.

The applicant also discussed the potential for proximal hive exposure. EPA is concerned with proximal hive exposure, wild pollinator exposure, and source hive exposure. According to the reported efficacy of *C. rosea* CR-7 during product testing, it is clear that the amount delivered to flowers is in a greater amount than what would be delivered via other methods of application (e.g., sprays) (BVT, 2018). Thus there is a specific concern regarding potential hazard and increased exposure for the bee vectoring use of the product for the mentioned nontargets.

The applicant's rationale provides further citations and data on loss of spores resulting from bee flight. The rationale states and provides a citation supporting the argument that once a bee visits a flower that other individual bees will not visit that flower, meaning that flowers visited by source bees carrying CR-7 will not be followed by bees from proximal hives. This statement is supported by only one citation and after internal deliberation it is not the opinion of EPA that this is accurate. Additionally, other pollinators besides bees may visit flowers already visited by source bees and be exposed.

The rationale concludes by explaining that a bee's spore carrying capacity for CR-7 upon exit from a hive is 4×10^4 CFU/bee and that this amount decreases with successive flights depending on how many flowers are visited, the morphology of the flower, and environmental degradation of the spores. An estimate was given that 1.62×10^2 CFU/bee at a maximum could enter a proximal hive. This estimate is based on the worst-case scenario where a source bee would visit a flower which would then be immediately visited by a bee from a proximal hive carrying the spores back to a proximal hive. This estimate could only be confirmed via testing using a pollen trap in proximal hives and an enumeration of CR-7 spores in the pollen trap. The applicant also stated that *C. rosea* is present in dry soil at 4×10^3 CFU/g and at 2×10^3 CFU/g on strawberries and that bees are consistently exposed to these amounts in nature and agroecosystems. While EPA agrees with this particular point, since the bee vectoring use pattern is intended to deliver higher amounts to flowers than would be expected from natural populations, source bee exposure would have to be higher than levels that would occur in nature. Therefore, unless exposure to proximal hives can be shown to be equal to natural levels of C. rosea in the environment, and source hives are destroyed after use such that effects on brood are not relevant, EPA will require larval testing.

References

Ahmed, M., M. Laing and I. Nsahlai. 2014. Use of Clonostachys rosea against sheep nematodes developing in pastures. Biocontrol science and technology 24: 389-398.

Bee Vectoring Technology. 2018. http://www.beevt.com/crops-we-help/strawberries-botrytis-cinerea/

Hamiduzzaman, M.M., A. Sinia, E. Guzman-Novoa and P.H. Goodwin. 2012. Entomopathogenic fungi as potential biocontrol agents of the ecto-parasitic mite, Varroa destructor, and their effect on the immune response of honey bees (Apis mellifera L.). Journal of invertebrate pathology 111: 237-243.

Organization for Economic Cooperation and Development (OECD). 2016. Guidance Document on Honey Bee Larval Toxicity Test following Repeated Exposure. ENV/JM/MONO(2016)34; July 15, 2016. Available at

http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2016)34&doclanguage=en.

Tedford, S. 2017. Bee Vectoring Technology Inc. Response to EPA 75-day Deficiency Letter for *C. rosea* CR-7. MRID 50477501.

U.S. EPA. 2017a. 75-day Deficiencies: Nontarget Organisms (40 CFR § 158.2150) and Label Deficiencies. Letter from Jeannine Kausch (EPA) to Jacob Moore (TSG Inc.), dated November 17 ,2017.

U.S. EPA. 2017b. Ecological Review and Environmental Risk Assessment of Section 3 Registration of the New End Use Product Vectorite containing *Clonostachys rosea* CR-7 vectored by honey and bumble bees. Memorandum from Milutin S. Djurickovic (EPA) to Nicola Steinmetz (EPA), dated September 25, 2017.

Vega, F.E., F. Posada, M.C. Aime, M. Pava-Ripoll, F. Infante and S.A. Rehner. 2008. Entomopathogenic fungal endophytes. Biological Control 46: 72-82.

Zhang, L., J. Yang, Q. Niu, X. Zhao, F. Ye, L. Liang, et al. 2008. Investigation on the infection mechanism of the fungus Clonostachys rosea against nematodes using the green fluorescent protein. Applied microbiology and biotechnology 78: 983-990.